USING ADVANCED HYBRID STOCHASTIC METHODS TO DESIGN BIOLOGICAL GENE NETWORKS THAT RAPIDLY RESPOND TO HARMFUL SUBSTANCE DETECTION

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Force protection during both peacetime and combat is extremely important to the safety and efficiency of the Army. Currently, the detection of harmful biological organisms involves microbiological culturing and biochemical tests that require at least three hours and access to a laboratory. Chemical tests for the detection of harmful chemical toxins are faster, but still require a mobile laboratory. On the battlefield, access to a laboratory may be limited and timing is critical. The method of detecting harmful substances must be fast and highly portable, capable of being carried by every soldier. By increasing the number of detectors on the battlefield, one increases the chance of successfully detecting a harmful substance. However, detectors that use biochemical tests, such as Real-Time PCR, to monitor for biological agents will always be limited to mobile laboratories, operated by trained biochemists.

Biological organisms, on the other hand, are able to innately interact with the chemical world and specifically distinguish harmful chemicals or biological organisms, such as Sarin or Anthrax. Biological organisms possess an internal analog signal processor, its regulated gene expression, that responds to environmental stimuli and produces a programmed response. By harnessing a biological organism's ability to interact with specific harmful substances, detect their presence, and produce a programmed response (such as fluorescence or color change), we can engineer organisms to become detectors possessing extraordinary sensitivity and specificity. One benefit to the usage of biological harmful substance detectors is that they self-replicate, operate independently, and may be deployed prior to any soldier setting foot on the ground. For example, by engineering harmless soil bacteria to fluoresce when in contact with a harmful chemical toxin, such as Sarin, one can deploy the bacteria onto suspected enemy installations and determine whether the toxin is present, perhaps in quantities that are extremely minute, but indicative of storage or other uses.

Engineering biological detectors requires three separate subsystems: an engineered receptor to bind specifically to the desired substance, a signal transduction pathway that conveys this binding event and produces an input signal, and an engineered gene network that accepts the input signal and rapidly produces a macroscopically detectable output signal, such as fluorescence or color change. Research on engineering receptors has been progressing with success[1]. We intend to focus on the latter subsystem; that is, we will design systems of interconnected genes, or gene networks, that, when given an input signal, rapidly respond and produce fluorescence. In order to design the gene network, we accurately represent the numerous interactions between regulatory proteins, DNA, and RNA as a system of coupled chemical and biochemical reactions. We then use stochastic methods to predict the stochastic dynamics of the system and use those predictions to guide the experimental implementation of the gene network into a

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bacterial organism. By using computational simulations, we can explore the large number of possible designs much more quickly than an experimentalist could by trial and error. Further, by exploring the trends in the predictions, we develop design principles that help to explain why natural gene networks are structured and how we can better engineer novel ones.

We have already computationally designed an improved gene network, called a bistable switch[2], that consists of two genes each producing a different repressor that mutually repress one another. The network may stably exist in either the 'off' or 'on' states and the state may be changed from 'off' to 'on' and vica versa by adding inducer molecules. By using self-repression to limit the steady-state concentrations of the repressor species, the improved bistable switch is predicted to switch 70% faster than its predecessor[3]. Using multiple operator DNA binding sites and DNA looping, the improved network has a larger difference between the analog signals of 'off' and 'on', enabling one to be more certain as to whether the switch is in the 'off' or 'on' position. One could use the bistable switch to turn on the production of fluorescence when the input signal, an inducer molecule, is produced via the signal transduction pathway in response to a receptor binding to a harmful substance.

In addition, we have improved upon existing simulation methodology by creating a hybrid stochastic method[4] that is capable of more quickly simulating a system of coupled chemical or biochemical reactions when one or more of those reactions occurs frequently. Depending upon the rates of the 'fast' reactions, the speed-up may be numerous orders of magnitude as compared to the commonly used stochastic simulation algorithm[5]. In brief, the method partitions the system into 'fast' and 'slow' reactions, mathematically describes time-evolution of the fast reactions using the chemical Langevin equation[6], and monitors the slow reactions using a system of integral algebraic equations, called Jump Equations, that have important properties allowing one to numerically solve them quickly and accurately. The hybrid stochastic method will be useful in designing gene networks that contain one or more fast reactions.

By designing gene networks that quickly respond to input signals and produce detectable output signals, we will produce a necessary subsystem of an engineered biological organism that detects the presence of harmful chemical or biological substances. Future work will involve designing biological analog filters that reduce possible false negatives or positives.

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